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Effect of total phenolic content and proline content on the resistant and susceptible chilli varieties influenced by root-knot nematode, *Meloidogyne incognita*

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Abstract
Proline metabolism has complex roles in a variety of biological processes, including cell signaling, stress protection, and energy production. Plant-derived phenolic compounds contribute to the defense against various pathogens, including plant parasitic nematodes. To investigate the role of proline and phenols in resistant and susceptible varieties of chilli against *Meloidogyne incognita* the fifteen days old chilli plants were inoculated with 1000 second stage juveniles of root knot nematode, *Meloidogyne incognita* and maintained under net house condition. Investigations were made to find out the effect of *Meloidogyne incognita* on the biometric traits and biochemical traits determining chlorophyll content, sugar content, starch content, phenol content, proline content, macro and micronutrient status of chilli crop. Studies on total phenol in roots and shoots of root-knot susceptible and resistant chilli varieties revealed significantly higher level in roots and shoots of moderately resistant variety Debgarh Local as compared to highly susceptible variety Ghatikya. In Debgarh Local phenolic content was increased up to 68.71% in shoot part and 50.13% in root part. Ghatikya variety was shown lower rate of increase in phenolic content as 22.27% in shoots and 18.25% in roots. The shoots of the tested chilli varieties exhibit comparatively less proline content, whereas the roots exhibit higher levels. Debgarh Local was shown maximum increase in proline content with 32.05% in roots and 29.46% in shoots and Ghatikya was shown minimum increase rate in proline content with 24.24% in roots and 16.66% in shoots.

Keywords: *Meloidogyne incognita*, phenolic content, proline, chilli cultivars, resistant and susceptible

Introduction
Chilli (*Capsicum annuum* L.) is an important vegetable cum spice crop valued for its aroma, taste, flavour and pungency, grown in all parts of the world. India is the world's largest producer, consumer and exporter of chilli in world. The secret to a good crop of chillies is a long, hot growing season. Nematodes are microscopic round worms that are common in this kind of climate. Root knot nematodes (*Meloidogyne* species), are the most prevalent and destructive of all plant-parasitic nematodes. Chilli being an important spice crop is attacked by several plant parasitic nematodes. In tropical and sub-tropical areas the most commonly occurring species is *Meloidogyne incognita* among all other nematodes. They are sedentary endoparasites that induce very complex tropic relationships with their host plants. An increase in phenolic compounds in early stage and later on it decreased (Bhau *et al.*, 2016) [2]. Singh (2013) [12] carried out quantitative analysis in bottlegourd and sponge gourd showing that proline, reducing sugars and free amino acids had a greater concentration in diseased roots over healthy one in both the host plants. Therefore, the present study was undertaken to find out the variation in phenolic substances and proline level of resistant and susceptible cultivars inoculated with *Meloidogyne incognita* in chilli cultivars.

Materials and Methods
Collection of nematodes
The root-knot nematode, *Meloidogyne incognita* was originally obtained from a single egg mass progeny, maintained and multiplied on susceptible brinjal variety Pusa Purple Long. The seeds of brinjal plants were sown in pots of 15 cm diameter containing sterilized soil, autoclaved at 15 lbs/ sq. inch pressure for 20 minutes. The populations of root-knot nematode were sub-cultured periodically inoculating with infective J_2 in sterile water suspension to the root zone of two week old seedlings grown in the pots.

Experimental procedure

When required plants were taken out from cultured pots, washed the roots free of soil, dissected out of the egg-masses from the galls under a stereoscopic microscope, treated with sodium hypochlorite solution. After that egg-masses washed with sterile water and put over tissue paper supported by aluminium wire gauge in petri dishes with water. J_2-stage hatched out was

collected daily in beakers and surface sterilized by treating with 0.5 percent streptomycin sulphate solution for 12 hours. Water from the upper portion of the beaker was drained off without disturbing the nematodes at the bottom. These larvae were utilized to conduct all the experiments. The larvae that could not be inoculated just after collection were stored in refrigerator at 8 to 10 °C for use within the next 3 to 4 days.

Earthen pots of 15 cm diameter, were sterilized with formaldehyde solution (1.0%) and filled with autoclaved soil (15 lbs/20min). These pots were arranged on greenhouse benches in complete randomized design with four replications.

For all chemical analysis purposes sterilized oven dried glassware and double distilled water were used throughout the experiment

Collection of chilli varieties/cultivars

Seeds of 10 chilli varieties/cultivars each of 2 to 3 gm, were collected from local market and farmer's field. The seeds were surface sterilized by treating them with 0.1% $HgCl_2$ for 5 minutes, washed thoroughly with sterile water and air dried. These seeds were sown in the pots to conduct the experiments.

Sowing of seeds and inoculation of nematodes

The soil to be filled in the pots were pulverized, mixed with fertilizers at the rate of 120 kg/hac for N, 80 kg/hac for P and 80 kg/hac for K and filled in the pots @ 1 kg/pot. The surface sterilized seeds were sown at the rate of 4 to 5 seeds per pot. Each variety was replicated 4 times. Moisture was maintained regularly after the emergence of seedlings. At 15 days after sowing the plants were thinned keeping one seedling per pot at the centre. A small glass tube (2 cm long, 0.5 cm bore) was inserted into the soil near the root zone of each of the seedlings. Two weeks after seedling emergence axenised nematodes were counted under a stereoscopic microscope and released into the holes near the root zones @ 1000 $J_2\pm$ 20 per seedling in 10 ml sterile water. For chemical analysis another two sets of plants were maintained, one for the uninoculated control (Healthy) and the other infected with the nematodes. Each set was arranged on separate platform in the green house in order to avoid cross infection.

Estimation of total phenolic substances

Exactly 0.1 g each of shoot and root sample was ground with a pestle and mortar in 10 ml of 80 percent ethanol until it became a pulp. The homogenate was centrifuged at 5000 rpm for 20 minutes. The process was repeated with another 5 ml of 80 percent ethanol. Both the supernatants were pooled and evaporated to dryness. The residue was dissolved in 10 ml distilled water. The aliquot was pipetted into test tubes with 0.5 ml each. The volume was made up to 3 ml with distilled water. Exactly 0.5 ml of folinciocalteu reagent was added into it. After 3 minutes 2 ml of 20 percent Na_2CO_3 solution was added into each tube. The contents were mixed thoroughly, placed in boiling water for 1 minute and then cooled. Absorbance was measured at 650 nm in a colorimeter and compared with a blank. A standard curve was prepared using different concentrations of catechol.

The concentrations of the phenol in test samples was calculated by comparing with the standard curve and expressed as mg/g material (catechol).

Estimation of total proline substance

Exactly 100 mgs each of shoot and root was macerated with 5 ml of sulfo-salicylic acid. The residue was centrifuged at 4000 rpm for 15 minutes. The supernatant liquid was decanted to a 50 ml test tube.5 ml of glacial acetic acid and 5ml of acid ninhydrin was added to it. The mouth of the test tube was closed by polythene paper and rubber band. It was boiled for 1hr. in water bath at 100 °C. After boiling of standards and sample, the reaction mixture was transferred to 60 ml separating funnels. 20ml of toluene was added and shaken vigorously. It was then allowed to settle.

The chromophore containing toluene was separated out through the bottom hole of the separating funnels. Absorbance was measured at 520 nm. By the help of standard curve data, the amount of proline present in plant sample was calculated and expressed as mg proline /gram of fresh sample.

Results and Discussion

In the present study, there were significant increment noticed among the chilli varieties for phenolic content against root knot nematode (*M. incognita*) infection (Table 1). The lower rate of increase in phenolic content was noticed in highly susceptible varieties. The results of this study revealed that resistant varieties shows increased rate of total phenolic content in both shoot and root parts.

The phenolic content of healthy shoots of chilli cultivars were 0.28, 0.36, 0.26, 0.17, and 0.23 mg/g and in healthy roots 0.37, 0.42, 0.34, 0.26, and 0.25 mg/g in Debgarh Local, Utkal Ava, G4, Surjomukhi Lanka and Ghatikya cultivars, respectively. Moreover, due to infection of root knot nematode the phenolic contents of these varieties increased by 68.71%, 66.76%, 45.42%, 29.09% and 22.27% in shoots respectively in the above said varieties (Table1).

The amount of phenol present in the roots of the uninoculated plants were recorded as 0.37, 0.42, 0.34, 0.26 and 0.25 mg/gm in the varieties Debgarh Local, Utkal Ava, G4, Surjomukhi Lanka and Ghatikya, respectively on fresh weight basis. Conversely this amount was increased in all the cases in inoculated plants ie., 0.56, 0.62, 0.46, 0.31 and 0.30 mg/g in the roots of these varieties. The results thus obtained were in agreement with the findings of Indurani *et al.* (2008) [5]; Ahmed (2012) [1]; Das *et al.* (2014) [4] and Nayak (2015) [8]. They also reported higher phenol content in nematode resistant plants than susceptible ones. Pandey *et al.* (2016) [10] reported that the phenolic content in healthy and infected green gram varieties ranged from 0.13 to 0.72 µg/g and 0.32 to 0.97 µg/g, respectively.

Effect of nematode infection on proline contents of chilli varieties

Results showed that *Meloidogyne incognita* feeding on chilli roots significantly changes the level of proline content in leaves as well as in chilli roots (Tables 2). This is consistent with the findings of other workers using root knot nematode and their corresponding host plant (Singh, 2013; Pandey *et*

al., 2016) [12, 11].

Proline level in roots and shoots of inoculated plants was increased when compared to uninoculated or healthy plants. The proline content of healthy shoots of chilli cultivars were 0.11 mg, 0.14 mg, 0.10 mg, 0.08 mg and 0.06 mg/g in Debgarh Local, Utkal Ava, G4, Surjomukhi Lanka and Ghatikya, respectively. Moreover, due to infection of root knot nematode the proline contents of these varieties increased by 29.46%, 28.57%, 24.48%, 18.18% and 16.66% in shoots respectively in the above said varieties (Table 2). Moderately resistant varieties contain the higher amount of proline than the susceptible and highly susceptible varieties. The minimum increase in shoot proline content was shown in the highly susceptible varieties Ghatikya and Surjomukhi Lanka followed by susceptible variety G-4. Shoots of inoculated chilli varieties shown comparatively less increase in proline content, whereas the roots exhibit significantly higher increase in proline contents. Moderately resistant variety Debgarh Local was shown maximum increase in root proline content with 32.05% and highly susceptible variety Ghatikya was shown minimum increase rate in root proline content with 24.24%.

Considering the widespread occurrence of root-knot nematode, *Meloidogyne incognita* and its pathogenic potential on various crops, the present investigations showed the significant changes in biochemical traits of all infected cultivars due pathogenicity of *M. incognita*. Under stress conditions increased activity of phenolic and proline content plays a pivotal role in defense mechanism. The total phenolic and proline content of the shoots and roots increases significantly in the most infected varieties compared to non-infected varieties. Understanding the mechanisms of how pathogens utilize proline is important for developing new strategies against infectious diseases

Table 1: Total Phenolic Content (mg/g) in the resistant/susceptible Chilli varieties influenced by root knot nematode, *Meloidogyne incognita.*

Sl. No.	Varieties	Phenol content (mg/g) on fresh weight basis							
		Shoot (leaf)		Mean	Increase/ decrease over healthy (%)	Root		Mean	increase/ decrease over healthy (%)
		Infected (I)	Healthy (H)			Infected (I)	Healthy (H)		
01	Debgarh Local (MR)	0.47	0.28	0.37	+68.71	0.56	0.37	0.46	+50.13
02	02 Utkal Ava (MR)	0.61	0.36	0.48	+66.76	0.62	0.42	0.52	+44.71
03	G4 (S)	0.38	0.26	0.32	+45.42	0.46	0.34	0.40	+33.23
04	Surjomukhi Lanka (HS)	0.21	0.17	0.19	+29.09	0.31	0.26	0.29	+20.38
05	Ghatikya (HS)	0.28	0.23	0.25	+22.27	0.30	0.25	0.27	+18.25
	SE(m)+	0.002	0.002			0.002	0.002		
	CD(0.05)	0.006	0.004			0.007	0.004		

Moderately Resistant (MR), Susceptible (S), Highly Susceptible (HS

Table 2: Total Proline Content (mg/g) in the resistant/susceptible chillvarieties influenced by root knot nematode, *Meloidogyne incognita*.

SI. No.	Varieties	Proline content (mg/g) on fresh weight basis							
		Shoot (leaf)		Mean	Increase/ decrease over healthy (%)	Root		Mean	Increase/ decrease over healthy (%)
		Infected (I)	Healthy (H)			Infected (I)	Healthy (H)		
01	Debgarh Local (MR)	0.14	0.11	0.13	+29.46	0.10	0.08	0.09	+32.05
02	Utkal Ava (MR)	0.18	0.14	0.16	+28.57	0.12	0.09	0.09	+29.67
03	G4 (S)	0.12	0.10	0.11	+24.48	0.10	0.08	0.06	+27.63
04	Surjomukhi Lanka (HS)	0.09	0.08	0.08	+18.18	0.06	0.04	0.02	+25.00
05	Ghatikya (HS)	0.07	0.06	0.06	+16.66	0.04	0.03	0.03	+24.24
	SE(m)+	0.003	0.002			0.012	0.001		
	CD(0.05)	0.009	0.007			0.036	0.004		

Moderately Resistant (MR), Susceptible (S), Highly Susceptible (HS)

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